A THESIS FOR THE DEGREE OF MASTER

# Efficacy and safety of canine adipose tissue-derived mesenchymal stem cells for treating insulin-dependent diabetes mellitus in dogs

# 인슐린 의존성 당뇨환견에서 개 지방유래 중배엽줄기세포 치료의 효과 및 안전성

2019년 8월

서울대학교 대학원
수의과대학 수의내과학 전공
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이 논문을 수의학석사학위논문으로 제출함

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# Abstract

## Efficacy and safety of canine adipose tissue-derived mesenchymal stem cells for treating insulin-dependent diabetes mellitus in dogs

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Mesenchymal stem cell (MSC) therapy is a promising alternative treatment for islet transplantation in patients with insulin-dependent diabetes mellitus (IDDM). Since MSCs possess regenerative and immunomodulatory properties, and are capable of controlling the immune dysregulation that leads to β-cell destruction, stem cell transplantation could be used in management of IDDM patients. However, in veterinary medicine, the application of canine stem cells to canine IDDM patients is not sufficient. In this study, we assessed whether canine adipose tissue-derived MSC (cAT-MSC) therapy could be an option for treatment of canine diabetes mellitus. With the written informed consent of the owner, canine IDDM patients who had received insulin treatment for more than 1 year at the Veterinary Medical Teaching Hospital of Seoul National University, were injected intravenously with allogenic cAT-MSCs five times

at 1-month intervals. During the treatment period, patients' clinical symptoms (weight loss, appetite, and polyuria and polydipsia), side-effects of stem cell treatment, and treatment efficacy (fasting C-peptide, triglyceride, total cholesterol, fructosamine, HbA1c, treated insulin dose, and urine test) were evaluated.

C-peptide was elevated by about 5–15% in 3 patients. Hyperlipidemia was resolved in 2 of 4 patients and 1 patient remained in the normal range. Fructosamine and Hb/A1c levels were improved in 2 patients. One patient showed improvement in proteinuria, 2 patients did not show proteinuria during the study.

Considering that C-peptide secretion capacity and lipid metabolism are related to diabetic complications, improvement in these two factors could imply that this treatment was effective in managing the risk of diabetic complications. This suggests that cAT-MSC therapy in diabetic patients may help to improve the insulin secretory capacity of IDDM patients and prevent diabetic complications.

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**keywords :** Insulin dependent diabetes mellitus, Canine adipose tissue-derived mesenchymal stem cell, Fasting serum C-peptide, Hyperlipidemia, Proteinuria,

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# 1. Introduction

Canine diabetes mellitus is a common disease, with a prevalence of 13 out of 10000 in a cohort study conducted in Sweden [13] and a 0.32% prevalence in one study conducted in the UK [17]. Of 860 patients diagnosed with diabetes in the Swedish study, the median survival time was 2 years, with the exception of 223 dogs who died at the time of diagnosis. In another study, the 1-year survival rate for canine diabetic patients was 64% [12] and diabetes commonly results in many complications [18, 31]. Considering those reports, diabetes mellitus is not only a common disease, but it also affects patients' length and quality of life.

Although 40% of diabetic patients die due to ketoacidosis or euthanasia on the day of diagnosis with diabetes mellitus [13], subsequent mortality could be due to diverse complications, including retinopathy, nephropathy, neuropathy, gastrointestinal, and infectious complications. These complications are known to be more likely to occur with a longer duration of diabetes [16, 34]. One study has shown that diabetic patients may have a higher prevalence of diabetic microvascular complications when their insulin secretion capacity is reduced; this can be evaluated by measuring fasting serum C-peptide levels [34, 35].

Most cases of diabetes mellitus in dogs are due to insulin-dependent diabetes mellitus, which is historically thought to be caused by pancreatitis [2, 20, 36, 41] or autoimmune-mediated beta cell destruction [3, 4, 9, 38], but one study contradicted commonly held concepts about autoimmune-mediated beta cell destruction [1]. However, this study was limited in that the study did not evaluate T cell-mediated autoimmune responses. In another study, although there was no direct evidence of B and T lymphocytic infiltration in the pancreatic tissue of the diabetic patients, except for early diabetic patients, only a few alpha, beta, PP, and ghrelin cells

remained after the extremely selective destruction of beta cells, suggesting that the possibility of autoimmune-mediated beta cell destruction was high [38]. Thus, autoimmune-mediated beta cell destruction is also likely to be one of the causes of canine insulin-dependent diabetes mellitus. Therefore, if MSCs are administered to patients with insulin-dependent diabetes mellitus, it is likely to lead to improvement of insulin secretion ability in diabetic patients [8, 19, 21, 43], given the immunomodulatory effect of MSCs [23, 25, 29, 33] and their potency for differentiation into insulin secretory cells [24].

Studies on stem cell therapy in diabetes have been conducted in humans and laboratory animals, and it has been reported that treatment with adipose tissue-derived MSCs or bone marrow-derived MSCs has been effective and safe [10]. However, there has been no study of the effect of canine adipose tissue-derived mesenchymal stem cell (cAT-MSC) therapy on dogs who have been treated for IDDM for more than 1 year. Therefore, this study investigated whether insulin secretion was improved when canine IDDM patients were treated with cAT-MSCs, and whether this treatment prevented or improved diabetic complications..

# 2. Materials and Methods

### *2.1. Patient selection*

Patients (Table 1) that had already been diagnosed with IDDM at Seoul National University Veterinary Medicine Teaching Hospital (SNU-VMTH) were enrolled in the study with their owner's consent. The inclusion criterion was that patients had been diagnosed with IDDM for more than 1 year. All owners received an explanation of the study in oral and written form, and owners gave written consent for participation before the study commenced. This study protocol and design were approved by the Seoul National University Institutional Animal Care and Use Committee (IACUC) and ethical approval has been granted (SNU-180321-4)

### *2.2. Study Design*

This study was a pilot study to evaluate the safety and efficacy of using cAT-MSCs for patients with type 1 diabetes who have been treated with insulin at SNUVMTH for more than 1 year. Patients who met the inclusion criterion were treated with cAT-MSCs and the therapeutic effect was assessed using the following evaluation criteria. The evaluation tests included changes in overall clinical symptoms, laboratory tests (fasting C-peptide, TG, total cholesterol, fructosamine, HbA1c, treated insulin dose, and urine test) and changes in required insulin dosage. The end of the study was 1 month after the completion of stem cell treatment, and the patients were evaluated every 1–2 months until that time-point.

### *2.3. Isolation, culture, characterization and injection of cAT-MSCs*

Canine adipose tissue for stem cell isolation was obtained from healthy 4-year-old puppies. The procedure was established with the approval of the IACUC, and all dogs were negative for major infectious diseases: canine parvo virus, canine coronavirus, and canine distemper virus.

cAT-MSCs were cultured after isolation, according to previously described methods [26]. First, adipose tissue was washed 5 times with a mixture of Dulbecco's Phosphate-Buffered Saline (DPBS, PAN-Biotech, Aidenbach, Germany) and 1% penicillin–streptomycin (PS; PAN-Biotech). Then, the tissue was cut into small pieces with sterile scissors. Physically excised samples were digested with collagenase type IA (0.1%, Gibco/Life Technologies, Carlsbad, CA, USA) for 60 min at 37°C and were then neutralized in a mixture of 10% fetal bovine serum (FBS, PAN-Biotech) and Dulbecco's Modified Eagle's Medium (DMEM, PAN-Biotech). The neutralized adipose tissue mixture was centrifuged at 1,200 × g for 5 minutes and then the undigested fragments were removed using a 70-μm cell filter (Thermo Fisher Scientific, Rockford, IL, USA). The obtained cells were resuspended in a mixture of 10% FBS, 1% PS, and DMEM and then cultured at 37°C and 5% $CO^2$ at a density of 3000/$cm^2$. Cell growth plates were washed with DPBS after 5 days to remove unattached cells and then further cultured in fresh medium. The culture medium was newly replaced every 2–3 days until cell confluency reached 70–80%. Then, the plate was subcultured at a density of 10,000/$cm^2$.

The cells were evaluated for stem cell marker expression using flow cytometry prior to the injection experiment. The identified stem cell markers were Clusters of Differentiation (CD) 29-fluorescein isothiocyanate (FITC), CD33-FITC, CD45-FITC, CD34-phycoerythrin (PE), CD73-PE (BD Biosciences, San Diego, CA, USA), and CD90-allopicosin (eBiosciences, San

Diego, CA, USA). Cell fluorescence analysis was performed with a FACS Aria II system (BD Biosciences). These cells were evaluated for their differentiation potential using stempro adipogenesis, osteogenesis, and chondrogenesis differentiation kits (Gibco, Grand Island, NY, USA). The differentiated cells were stained with Oil Red O, Alizarin Red, and Alcian Blue, respectively.

Patients were treated with chlorpheniramine (0.5 mg/kg, intravenously) 15 minutes before stem cell treatment to prevent hypersensitivity reaction. P4 cAT-MSC ($5 \times 10^6$ cells/kg) diluted with a sufficient volume of 0.9% saline was injected intravenously over a period of 30 minutes.

### *2.4. Safety Evaluation*

History was taken and laboratory evaluations performed to monitor adverse events. This included the incidence of hypersensitivity, inflammation, or other conditions around the injection site, neoplasm, and severe hypoglycemia or hyperglycemia. On the day when the stem cells were administered, the hypersensitivity reaction was monitored through evaluating body temperature, heart rate, blood pressure, and injection site observation for 30 minutes after MSC administration. When the patient visited for the next stem cell treatment, we asked the owner about the history of pain at the site after treatment and about side effects suggesting hypoglycemia at home during the previous 1 month. Then, a general physical examination was performed. On the day of treatment, we also measured fasting blood sugar levels to determine whether serious hyperglycemia or hypoglycemia had occurred.

### *2.5. Efficacy Tests*

Treatment efficacy evaluation included clinical sign monitoring, fasting C-peptide, HbA1c, fructosamine, TG, total cholesterol, treated insulin dose, and urine test evaluations. Monitoring these profiles started after deciding on stem cell therapy..

#### *2.5.1 Clinical sign monitoring*

The general clinical symptoms were assessed for changes in body weight, vitality, appetite, and water intake. Vitality was evaluated using a 5-point system: 5. Patient walks lightly, actively responds to external stimuli, including the owner's call, and there is little change in sleeping time. 4. There is no change in response to walking and external stimuli, but the sleeping time is greatly increased. 3. Patient does not move when walking and the response to external stimuli is poor. 2. Moves only for defecation or urination. 1. Patient cannot move and defecates and urinates in the lying position.

The appetite was also evaluated using a 5-point scale: 5. Eats all of the recommended food in 1 sitting. 4. Eats the recommended amount of food, but does not eat it in 1 sitting. 3. Does not eat the recommended amount of food, but eats more than half. 2. Leaves at least half of the recommended amount of food. 1. No appetite. The amount of water intake was evaluated based on the owner's observation using a 120-ml cup. We considered that PU/PD occurred if the patient’s water intake was above 100 ml/kg/day [11, 14, 32]. In addition, to evaluate the neurological complications caused by diabetes mellitus, we evaluated tachycardia due to increased sympathetic tone or hypotension due to decreased sympathetic tone [16, 18, 31].

### *2.5.2 Fasting C-peptide monitoring*

To evaluate the insulin secretion ability of the beta cells [35] and the possibility of complications caused by diabetes mellitus, we measured the patient's fasting C-peptide levels [34, 40]. We collected blood samples when the patients visited after 12 hours' fasting. The serum samples were centrifuged and stored at -80 °C until the last MSC treatment. The serum samples were then thawed at room temperature and analyzed using a Dog Insulin C-Peptide ELISA kit (LS Bio, Inc, Seattle, WA, USA).

### *2.5.3 Blood analysis*

Fructosamine, HbA1c, TG, and total cholesterol levels were evaluated by blood analysis. Blood samples were collected after 12 hours' fasting. Those results showed the patient's blood glucose management status during the study and helped to estimate the possibility of diabetes complications [6, 15, 16, 18, 22, 31, 34, 42].

### *2.5.4 Urine analysis*

A urine test was performed to monitor proteinuria [30] and bacterial cystitis [28], which are common complications in diabetic patients. The urine samples were taken by cystocentesis and was used in a urine dipstick test (Combur-Test strips, Roche, Basel, Switzerland) and urinary sediment test. When proteinuria was found by the urine dipstick test, a urinary sediment test was performed to evaluate the presence of cystitis, followed by quantitative analysis of urine

protein–creatinine ratio.

# 3. Results

### *3.1. cAT-MSC differentiation*

cAT-MSCs used in this study were successfully differentiated into adipocytes, osteocytes, and chondrocytes. Each cell-type was stained with specific dyes that allowed their identification by microscopic evaluation (Fig. 1).

### *3.2 Safety evaluation*

During the 18 times administration of cAT-MSCs, no side effects were observed other than in a patient who showed pain at the injection site on the day of treatment and a flare at the injection site up to the next day, once, after the $5^{th}$ injection.

### *3.3 Clinical sign monitoring*

After stem cell treatment, 3 owners observed improvement of the respective patient's vitality (Table 2). No tachycardia or hypotension was observed on physical examination in any of the patients. Two of 4 patients showed improvement in appetite, polyuria and polydipsia (PU/PD), and weight; the weight of these patients increased by 6.83% and 8.69%, respectively. The other 2 dogs' body weight decreased by 2.1% and 6% during the treatment period.

### *3.4 Fasting C-peptide monitoring*

Fasting serum C-peptide levels were measured in the morning of each patient's visit (Fig. 2). C-peptide secretion was increased in 3 of 4 patients, while 1 patient showed decreased fasting serum c-peptide levels. Patients with a positive response to treatment showed improvement in C-peptide secretion after 1 or 2 stem cell treatments and improved 5%–15% compared with levels before stem cell treatment. During the treatment period, C-peptide of patient 1 increased from 3.02 pg/ml to 3.47 pg/ml, that of patient 2 increased from 3.35 pg/ml to 3.53 pg/ml, that of patient 3 increased from 3.46 pg/ml to 3.67 pg/ml, and that of patient 4 decreased from 3.55 pg/ml to 3.37 pg/ml.

### *3.5 Blood analysis and treated insulin dose*

During the study, fructosamine and glycated hemoglobin (HbA1c) decreased in 2 of 4 patients, while the levels in the other patients were unchanged or increased (Table 3). In patient 1, fructosamine decreased from 516 μmol/L to 354 μmol/L after treatment, and HbA1c decreased from 9.8% to 7.4%. The fructosamine concentration of patient 3 was decreased from 433 μmol/L to 301 μmol/L and the HbA1c levels did not change significantly. Insulin doses were reduced in 2 of 4, unchanged in 1, and increased in 1 patient. TG and total cholesterol levels were within the normal range [37] in 3 of 4 patients. Among the 3 patients, hyperlipidemia was resolved in 2 patients, while 1 of 4 patients showed increased serum TG levels, above the normal range (Fig. 3).

### *3.6 Urine analysis*

Two of 4 patients showed no evidence of proteinuria during this study, 1 showed improvement in proteinuria, and 1 did not have sufficient continuous evaluation data due to the owner's refusal for further examination (Table 4). None of the patients had bacterial cystitis.

# 4 Discussion

A previous study had shown that insulin secretory capacity remains present in IDDM patients who have been treated with insulin for a long period of time [40]. Therefore, the therapeutic effects of stem cells [23-25, 29, 33] can be expected not only to involve transplantation of new beta cells, but also preservation of remaining beta cells. It has been reported that the insulin secretory capacity was improved in diabetic patients as well as in laboratory diabetic model animals [10, 44] when AT-MSCs were administered via a venous or splenic route. However, to date, no studies had been performed in canine patients suffering from IDDM for more than 1 year. The present study examined whether cAT-MSC therapy in canine IDDM patients could be an option for diabetes treatment by preserving or improving pancreatic beta cell function.

During the course of treatment, the patient's vitality, inflammation near the injection site, occurrence of neoplastic mass, and hypoglycemia were examined, to evaluate safety of this treatment, by means of history taking, physical examination, and blood tests. cAT-MSCs were administered 18 times in total. No side effects were observed other than in a patient who showed pain at the injection site on the day of treatment and a flare at the injection site up to the next day, once, after the $5^{th}$ injection.

To evaluate the therapeutic effect, blood tests, including fasting C-peptide concentration [34, 40], serum TG, total cholesterol [18, 42], fructosamine, and Hb1Ac [6] evaluations were performed, and proteinuria and neurological diseases were evaluated as complications of diabetes mellitus [31]. The fasting C-peptide concentration increased in 3 of 4 patients. The C-peptide is an indicator of insulin secretion in pancreatic beta cells [35]. Therefore, the elevation of fasting C-peptide levels might suggest that the insulin secretory

capacity of these 3 patients may have been preserved or improved. In human studies, fasting plasma C-peptide levels were found to be lower with a longer duration of diabetes, and the lower the fasting C-peptide, the higher the TG level and the more frequently complications due to diabetes are reported [34]. Therefore, if there was an improvement in the lipid metabolism profile in those patients, the observed elevation in C-peptide could suggest that cAT-MSC treatment can help prevent diabetic complications.

Serum TG and total cholesterol were measured to assess fat metabolism. Diabetic patients are more likely to have hyperlipidemia due to aberrant fat metabolism [18, 42]. When diabetes is properly managed, hyperlipidemia improves [15, 22]. Generally, hyperlipidemia could cause pancreatitis, hepatobiliary disease, atherosclerosis, ocular disease, and seizures or other neurologic signs [22, 42]. Therefore, resolving hyperlipemia is an important point in managing the complications of diabetes. Patients with improved fructosamine resolved hyperlipidemia or maintained a normal range of serum TG and cholesterol. One of the 2 patients without improved fructosamine resolved severe hyperlipidemia and mild hypercholesterolemia. Patient 2, who was treated with a reduced dose of insulin due to the risk of hypoglycemia showed mild hyperlipidemia and hypercholesterolemia. The results suggest that patients treated with cAT-MSC may avoid complications due to hyperlipidemia because these patients, including the patient who showed mild hyperlipidemia, did not require medical treatment for hyperlipidemia [22].

Fructosamine and HbA1c are indexes of blood glucose management over the previous 2–3 weeks and previous 10–14 weeks, respectively [6]. Therefore, fructosamine may be effective for the evaluation of the therapeutic effect after 2–3 weeks of stem cell treatment, and Hb/A1c for evaluation after 10–14 weeks of stem cell treatment. However, these values can be affected by adjusting the amount of insulin treatment, according to the clinical symptoms, as

well as by the effect of stem cells [6]. Therefore, it is necessary to evaluate these along with changes in the amount of insulin treatment.

In patient 1, fructosamine decreased from 516 μmol/L to 354 μmol/L and HbA1c decreased from 9.8% to 7.4%, while insulin increased by 20% over the same period. Patient 2 had a risk of hypoglycemia, because fructosamine was 212 μmol/L in the pre-treatment evaluation. Thus, we reduced insulin from 0.73 U/kg to 0.63 U/kg during the treatment period and the patient's fructosamine increased from 212 μmol/L to 324 μmol/L and HbA1c increased from 4% to 6.5%. In patient 3, although we used an equal dose of insulin throughout the treatment period, fructosamine decreased from 433 μmol/L to 301 μmol/L and HbA1c decreased slightly from 7.4% to 7.3%. In Patient 4, fructosamine increased from 358 μmol/L to 448 μmol/L during the stem cell treatment period. However, we even reduced the insulin dosage due to the risk of hypoglycemia, considering that the blood glucose curve examination results showed a nadir under 80 mg/dL [5]. This patient showed paradoxical results of gradual weight loss, increased fructosamine, and consistent PU/PD occurring with a blood glucose curve indicating a risk of hypoglycemia. In this case, due to a too-low volume of undiluted glargine insulin (around 0.01 ml), it is possible that the owner was not able to take the appropriate dose at home, or that glargine insulin may have been overdosed while drawing the glucose curve in the hospital.

The urine test was performed to evaluate diabetic nephropathy. Nephropathy in diabetic patients is associated with glucose utilization at the cellular level and microvascular complications [27, 30, 31, 39]. The test included a dip-stick test and urine sedimentation test. In addition, urine protein–creatinine (UPC) ratio evaluation was performed when proteinuria without cystitis was detected by dip-stick test. Two of 4 patients showed evidence of dip-stick proteinuria, which was shown to be improved in the patient who underwent continuous UPC

assessment. Considering that 1 patient with proteinuria showed improvement and that no proteinuria occurred in 2 patients during 6 months, the results suggest cAT-MSC treatment could help patients prevent nephropathy complications due to IDDM.

The study had some limitations. It was conducted on 4 patients; this small sample size limited assessment of the statistical significance of the treatment effect. In addition, in order to evaluate insulin secretion, a continuous C-peptide measurement, using a mixed meal tolerance test or a 90-minute post-dietary test, has been established as a general evaluation method [7]. In this study, however, additional blood sampling was limited due to the owners' refusal. Thus, only fasting C-peptide levels could be evaluated [34]. Furthermore, although the association between diabetic microvascular complications (retinopathy, nephropathy, neuropathy) and fasting C-peptide concentration has been reported [16, 18, 31, 34] in a study of humans, systematic verification data are not available in veterinary medicine. Thus, considering that the fasting C-peptide concentration was used as an index of the association between diabetic complications and C-peptides in humans [34], we decided to use fasting C-peptide concentrations in this study.

In this study, 3 of 4 patients treated with cAT-MSCs showed improved fasting C-peptide secretion, while the study results indicate the possibility of the improvement and prevention of hyperlipidemia and proteinuria by MSC treatment. A previous study suggested that the lower the C-peptide secretion capacity and the higher the hyperlipidemia, the higher the probability of diabetic complications, suggesting that these two improvements (in C-peptide and hyperlipidemia) could imply that the treatment was effective in managing the risk of diabetic complications. Therefore, the results of this study suggest that cAT-MSC therapy could be one of the options for treating diabetes in dogs.

Table 1. Enrolled DM patients signalment

| | Age | Breed | Sex | BCS | DM duration |
|---|---|---|---|---|---|
| Patient 1 | 11 y | Toy poodle | MC | 5/9 | 1 y |
| Patient 2 | 15 y | Toy poodle | FS | 4/9 | 5 y |
| Patient 3 | 14 y | Pomeranian | FS | 4/9 | 2 y |
| Patient 4 | 11 y | Mongrel | MC | 5/9 | 1 y |

BCS, body condition score; DM, diabetes mellitus

Table 2. Clinical sign monitoring(Vitality, appetite, PU/PD, BW) results during cAT-MSC treatment

| | Treatment | Vitality | Appetite | PU/PD | BW | BP |
|---|---|---|---|---|---|---|
| Patient 1 | Pre-treatment | 5 | 4 | No PU/PD | 4.68 | 140 |
| | Post treatment #1 | 5 | 4 | PU/PD | 4.81 | 120 |
| | Post treatment #2 | 5 | 5 | PU/PD | 4.89 | 140 |
| | Post treatment #3 | 5 | 5 | PU/PD | 4.82 | 140 |
| | Post treatment #4 | 5 | 5 | PU/PD | 4.87 | 120 |
| | Post treatment #5 | 5 | 5 | No PU/PD | 5 | 130 |
| Patient 2 | Pre-treatment | 4 | 5 | No PU/PD | 2.76 | 110 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Post treatment #1 | 4 | 5 | No PU/PD | 2.88 | 130 |
| | Post treatment #2 | 3 | 5 | No PU/PD | 3 | 100 |
| Patient 3 | Pre-treatment | 4 | 4 | PU/PD | 2.40 | 140 |
| | Post treatment #1 | 5 | 5 | No PU/PD | 2.37 | 130 |
| | Post treatment #2 | 5 | 5 | NoPU/PD | 2.39 | 180 |
| | Post treatment #3 | 5 | 5 | NoPU/PD | 2.35 | 130 |
| | Post treatment #4 | 5 | 5 | NoPU/PD | 2.39 | 90 |
| | Post treatment #5 | 5 | 5 | No PU/PD | 2.35 | 110 |
| Patient 4 | Pre-treatment | 4 | 4 | PU/PD | 5.43 | 160 |
| | Post treatment #1 | 5 | 5 | PU/PD | 5.16 | 150 |
| | Post treatment #2 | 5 | 5 | PU/PD | 5.17 | 135 |
| | Post treatment #3 | 5 | 5 | PU/PD | 5 | 110 |
| | Post treatment #4 | 5 | 5 | PU/PD | 5 | 130 |
| | Post treatment #5 | 5 | 5 | PU/PD | 5.12 | 120 |

PU/PD, polyuria/polydipsia; BW, body weight; BP, blood pressure.

Table 3. Blood test results(Fructosamine, Hb/A1c, triglyceride, total cholesterol) and treated insulin dose during cAT-MSC treatment

| | Treatment | Fructosamine (μmol/L) | Hb/A1c (%) | Insulin dose (U/kg) | TG (mg/dL) | T. chol (mg/dL) |
|---|---|---|---|---|---|---|
| Patient 1 | Pre-treatment | 516 | 9.8 | 0.63 | 97 | 289 |
| | Post treatment #1 | 490 | 9.7 | 0.63 | 59 | 259 |
| | Post treatment #2 | 464 | 10.9 | 0.63 | 48 | 243 |
| | Post treatment #3 | 532 | 8.9 | 0.69 | 46 | 287 |
| | Post treatment #4 | 515 | 8.9 | 0.69 | 47 | 319 |
| | Post treatment #5 | 354 | 7.4 | 0.79 | 125 | 320 |
| Patient 2 | Pre-treatment | 212 | 4.0 | 0.73 | 129 | 387 |
| | Post treatment #1 | 287 | 6.6 | 0.65 | 97 | 423 |
| | Post treatment #2 | 324 | 6.5 | 0.63 | 219 | 423 |
| Patient 3 | Pre-treatment | 433 | 7.4 | 1.1 | 368 | 410 |
| | Post treatment #1 | 382 | 7.4 | 1.1 | 284 | 219 |
| | Post treatment #2 | 368 | 7.8 | 1.1 | - | - |
| | Post treatment #3 | 420 | 7.4 | 1.1 | 110 | 258 |
| | Post treatment #4 | 375 | 8.0 | 1.1 | 70 | 324 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Post treatment #5 | 301 | 7.3 | 1.1 | 131 | 251 |
| Patient 4 | Pre-treatment | 358 | 9.1 | 0.24 | 1203 | 355 |
| | Post treatment #1 | 417 | 9.1 | 0.21 | 54 | 274 |
| | Post treatment #2 | 425 | - | 0.19 | - | - |
| | Post treatment #3 | 338 | 9.9 | 0.20 | - | - |
| | Post treatment #4 | 492 | 11.2 | 0.20 | 51 | 263 |
| | Post treatment #5 | 448 | 8.7 | 0.22 | - | - |

Hb/A1c, hemoglobin A1c; TG, triglyceride; T. chol, total cholesterol.

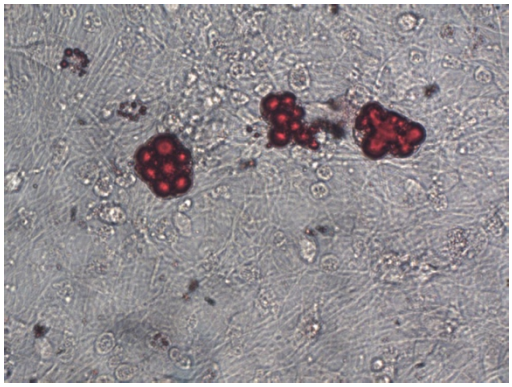
Table 4. Urinary test results(USG, diptsick, sediment, UPC) during cAT-MSC treatment

| | Treatment | USG | Dipstick | Sediment | UPC |
|---|---|---|---|---|---|
| Patient 1 | Pre-treatment | 1.037 | Glu 4+ | No inflammatory evidence | - |
| | Post treatment #1 | 1.051 | Glu 4+ | No inflammatory evidence | - |
| | Post treatment #2 | 1.042 | Glu 4+ | No inflammatory evidence | - |
| | Post treatment #3 | 1.039 | Glu 4+ | No inflammatory evidence | - |
| | Post treatment #4 | 1.050 | Glu 4+ | No inflammatory evidence | - |
| | Post treatment #5 | 1.051 | Glu 4+ | No inflammatory evidence | - |
| Patient 2 | Pre-treatment | - | - | - | - |
| | Post treatment #1 | 1.043 | Glu 4+ | No inflammatory evidence | - |
| | Post treatment #2 | - | - | - | - |
| Patient 3 | Pre-treatment | 1.018 | Glu 3+, Pro 2+ | No inflammatory evidence | 1.64 |
| | Post treatment #1 | 1.025 | Glu 3+, Pro 3+ | No inflammatory evidence | 3.23 |
| | Post treatment #2 | 1.010 | Glu 2+, Pro trace | No inflammatory evidence | 1.89 |
| | Post treatment #3 | 1.025 | Glu 3+, Pro 2+ | No inflammatory evidence | 1.13 |
| | Post treatment #4 | - | - | - | - |
| | Post treatment #5 | 1.014 | Glu 3+, Pro 2+ | No inflammatory evidence | 1.2 |

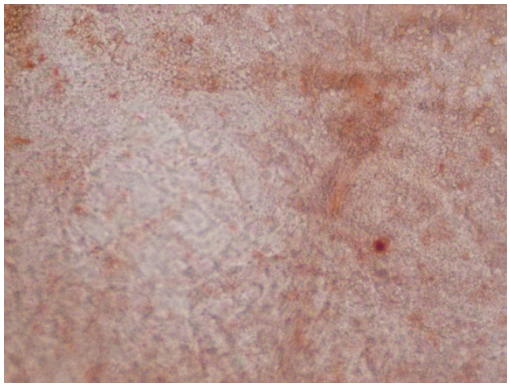
| | | | | | |
|---|---|---|---|---|---|
| Patient 4 | Pre-treatment | 1.018 | Glu 2+, Pro trace | No inflammatory evidence | - |
| | Post treatment #1 | 1.021 | Glu 4+, Pro trace | No inflammatory evidence | - |
| | Post treatment #2 | 1.020 | Glu 4+ | No inflammatory evidence | - |
| | Post treatment #3 | 1.023 | Glu 4+, Pro trace | No inflammatory evidence | - |
| | Post treatment #4 | - | - | - | - |
| | Post treatment #5 | - | - | - | - |

USG, urine specific gravity; UPC, urine protein creatinine ratio

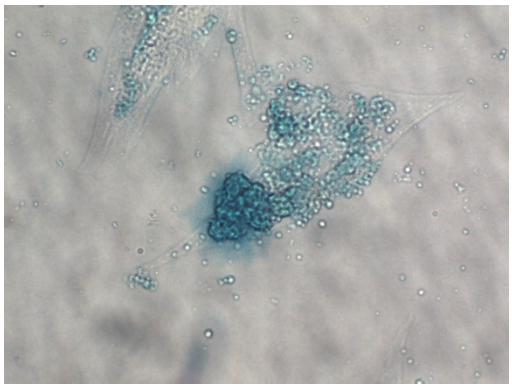
Figure 1. Adipocytes, osteocytes, chondrocytes differentiated from cAT-MSCs



(A) Adipocytes stained with Oil Red O.



(B) Osteocytes stained with Alizarin Red.



(C) Chondrocytes stained with Alcian Blue

Figure 2. Changes of fasting serum C- peptide in 4 patients during cAT-MSC treatment.

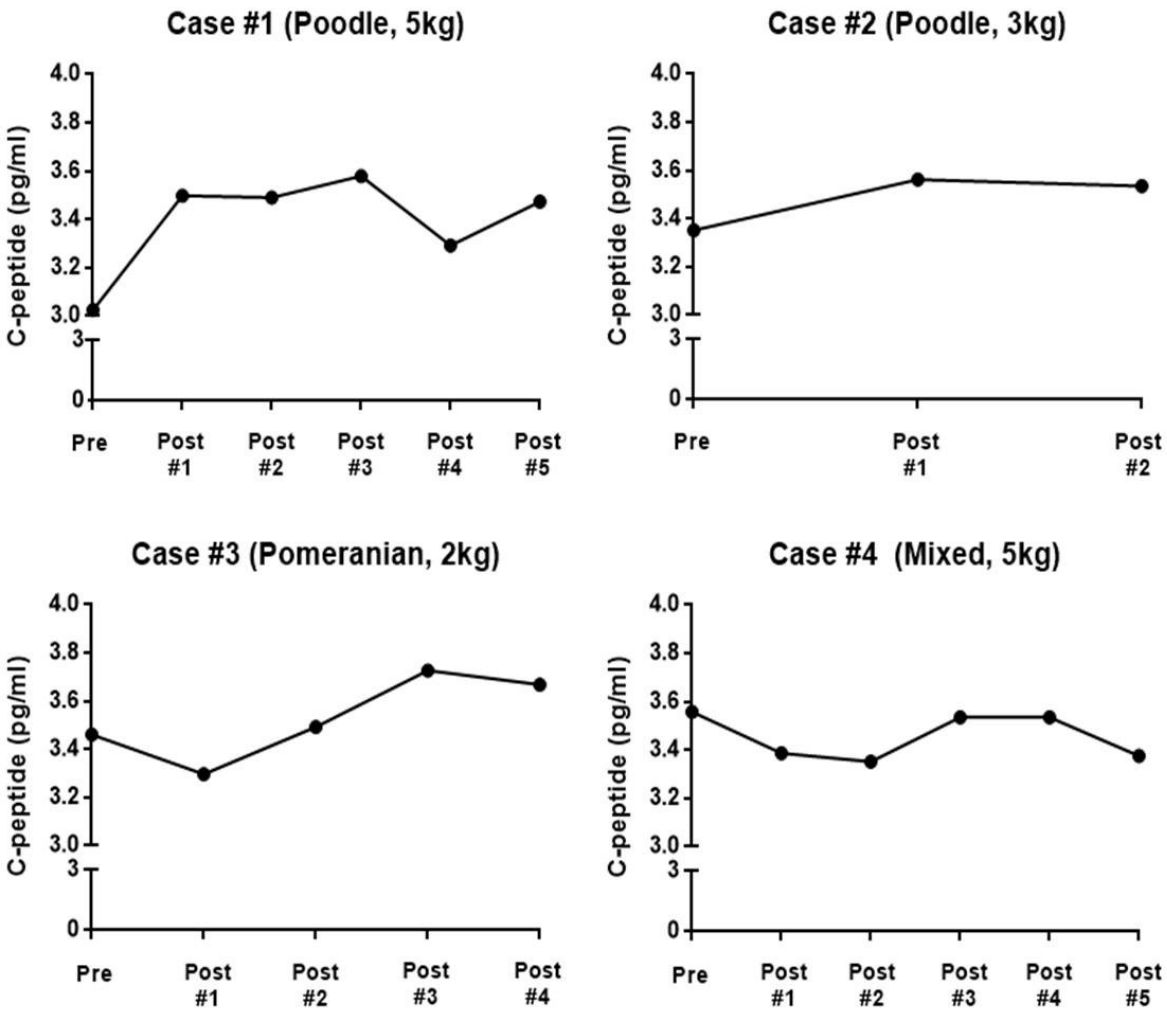
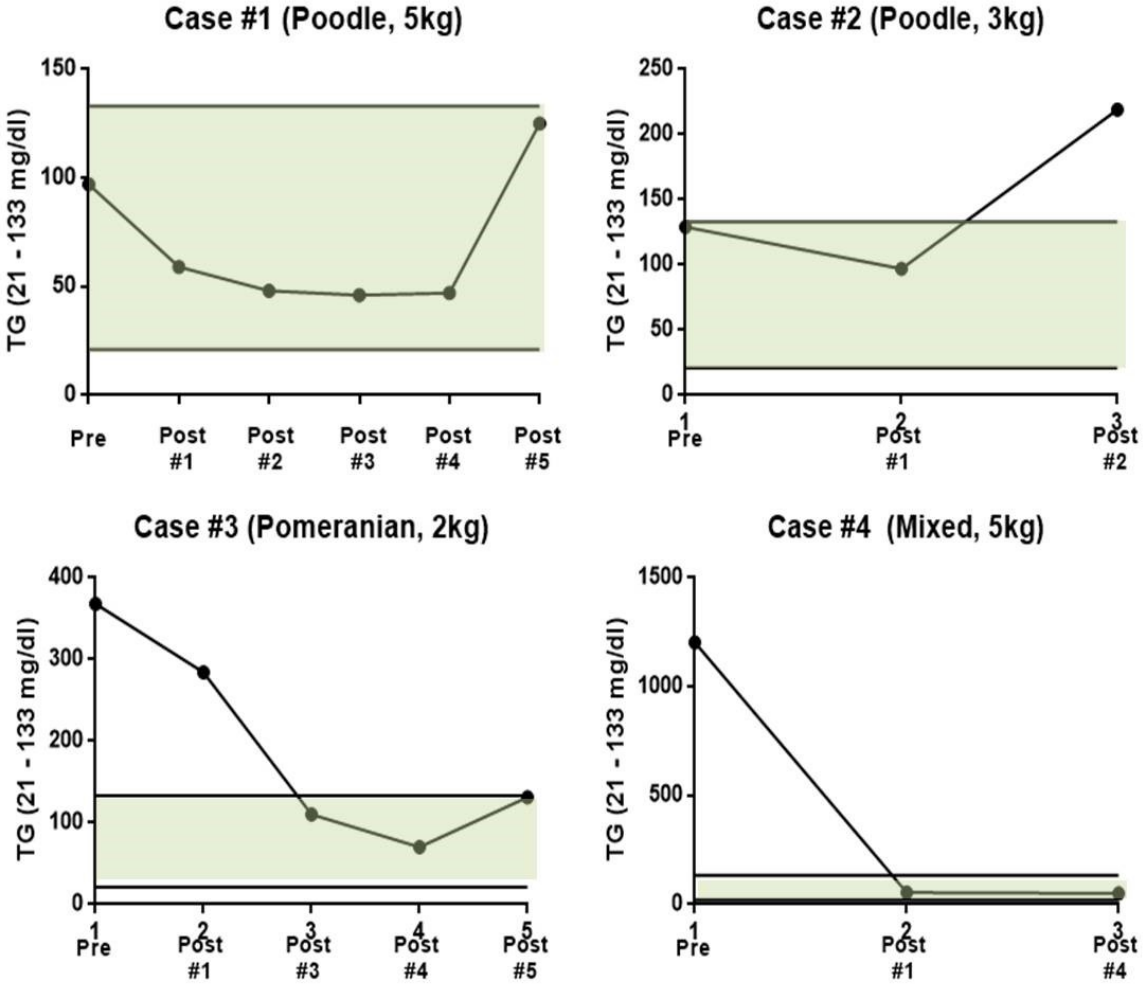


Figure 3. Changes of serum triglyceride in 4 patients during cAT-MSC treatment.




Reference range of serum triglyceride: 21-133 mg/dl

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# 국문초록

## 개 인슐린 의존성 당뇨환자에서의 개 지방조직 유래 중배엽줄기세포 치료의 효과 및 안전성

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류 성 용

중배엽 줄기세포(Mesenchymal stem cell, MSC)를 이용한 치료는 인슐린 의존성 당뇨병(Insulin-dependent diabetes mellitus, IDDM) 환자에게 시술할 수 있는 췌도이식술 대체를 목적으로 지속적으로 연구되고 있는 대안 중 하나이다. MSC는 베타세포 파괴를 유발시킬 수 있는 면역 교란 상태를 조정할 수 있는 면역 조절 특성과 세포 자체를 재생시킬 수 있는 특성을 갖고있어 IDDM 환자의 관리에 적용시켜볼 수 있다. 그러나 수의학 분야에서 개 줄기세포를 개 IDDM 환자에게 적용시킨 경우는 보고된 바가 드물다. 본 연구에서는 개 지방조직 중배엽 줄기세포(Canine adipose tissue derived mesenchymal stem cell, cAT-MSCs) 요법이 개 당뇨병 치료 방법 중 하나가 될 수 있을지 평가해보았다. 이를 위해서 서울 대학교 수의과 대학 병원에서 1 년 이상 인슐린 치료를 받은 4마리의 IDDM 개 환자

보호자의 서면 동의를 얻은 후, 동종 cAT-MSCs를 1 개월 간격으로 5 회 정맥 주사했다. 줄기세포 치료는 총 18회 진행되었으며, 환자 1, 3, 4는 각각 5번의 줄기세포 치료를 받았고, 환자 2는 3번의 치료를 받았다. 치료 기간 동안, 저자는 환자의 임상 증상 (체중 감소, 식욕 및 다음 다뇨), 줄기 세포 치료 및 치료 효능의 부작용 (공복 C-펩타이드, 중성 지방, 총 콜레스테롤, 프락토사민, 당화혈색소, 인슐린 투여량, 소변검사)에 대한 평가를 실시했다. 결과적으로 C-펩타이드는 3 마리 환자에서 약 5-15 % 증가하였고 4 마리 중 2 마리에서 고지혈증이 치료되었고 1 마리는 혈중 중성 지방이 정상 범위를 유지했다. 프락토사민과 당화혈색소는 2 마리의 환자에서 개선을 보였으며 그 2 마리의 환자 중 한 마리는 인슐린 투여량 변화 없이 개선을 보였다. 한 마리의 환자는 단백뇨가 호전되었고, 두 마리의 환자는 단백뇨가 없는 상태를 유지했다. C-펩타이드 분비능 및 지질 대사가 당뇨병 합병증과 관련되어 있다는 것을 고려하면, 이 결과를 통해 해당 치료가 당뇨병 합병증의 위험을 관리하는데 유효한 방법 중 하나가 될 수 있음을 알 수 있다. 따라서 본 연구를 통하여 당뇨병 환자에서 cAT-MSC 치료가 IDDM 환자의 인슐린 분비 능력을 향상시키고 당뇨병으로 인한 합병증을 예방하는 데 도움이 될 수 있음을 확인할 수 있었다.

**주요어 : 인슐린 의존성 당뇨, 개 지방유래 중배엽 줄기세포, 공복 혈청 C-펩타이드, 고지혈증, 단백뇨**

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